Title

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Journal

The Journal of cell biology, 124(6)

ISSN

0021-9525

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Publication Date

1994-03-01

DOI

10.1083/jcb.124.6.1039

Peer reviewed

Expression of a β_1-Related Integrin by Oligodendroglia in Primary Culture: Evidence for a Functional Role in Myelination

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Abstract. We have investigated the expression of integrins by rat oligodendroglia grown in primary culture and the functional role of these proteins in myelinogenesis. Immunochemical analysis, using antibodies to a number of α and β integrin subunits, revealed that oligodendrocytes express only one detectable integrin receptor complex ($\alpha_{OL}\beta_{OL}$). This complex is immunoprecipitated by a polyclonal anti-human β_1 integrin subunit antibody. In contrast, astrocytes, the other major glial cell type in brain, express multiple integrins including $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$ complexes that are immunologically and electrophoretically indistinguishable from integrins expressed by rat fibroblasts. The β subunit of the oligodendrocyte integrin (β_{OL}) and rat fibroblast β_1 have different electrophoretic mobilities in SDS-PAGE. However, the two β subunits appear to be highly related based on immunological cross-reactivity and one-dimensional peptide mapping. After removal of N-linked carbohydrate chains, β_{OL} and β_1 comigrated in SDS-PAGE and peptide maps of the two deglycosylated subunits were identical, suggesting differential glycosylation of β_1 and β_{OL} accounts entirely for their size differences. The oligodendrocyte α subunit, α_{OL}, was not immunoprecipitated by antibodies against well characterized α chains which are known to associate with β_1 (α_3, α_4, and α_5). However, an antibody to α_8, a more recently identified integrin subunit, did precipitate two integrin subunits with electrophoretic mobilities in SDS-PAGE identical to α_{OL} and β_{OL}. Functional studies indicated that disruption of oligodendrocyte adhesion to a glial-derived matrix by an RGD-containing synthetic peptide resulted in a substantial decrease in the level of mRNAs for several myelin components including myelin basic protein (MBP), proteolipid protein (PLP), and cyclic nucleotide phosphodiesterase (CNP). These results suggest that integrin-mediated adhesion of oligodendrocytes may trigger signal(s) that induce the expression of myelin genes and thus influence oligodendrocyte differentiation.

MYELINATION is a major developmental process of the nervous system, carried out by oligodendroglia in the central nervous system (CNS),[1] and Schwann cells in the peripheral nervous system (PNS). Myelin is a membranous sheath that is an extension of the plasma membrane of the myelin producing cell. It consists of numerous alternating lipid and protein-containing lamellae wrapped tightly around a segment of neuronal axon, functioning as an insulator to accelerate the velocity of electrical impulses transmitted between a neuronal cell body and its target cell (Raine, 1984). The brain also contains numerous process-bearing cells including neurons that are not myelinated. Little is known about the biochemical processes underlying specific recognition between oligodendroglia and their neuronal targets and/or their surrounding extracellular matrix (ECM). Adhesion events are likely to be critical in determining the ability of oligodendroglia to form myelin. A number of adhesion molecules have been suggested to take part in myelination. In Schwann cells myelin-associated glycoprotein (MAG) and L1 have been implicated (for review see Quarles, 1989). In neurons, L1 and N-CAM are likely to be involved (Nieke and Schachner, 1985; Martini and Schachner, 1986).

In addition to cell–cell interactions it is now evident that cell–matrix interactions play a significant role in development. Integrins are a family of cell surface receptors which translate signals outside the cell to alterations in cell behavior. A role for integrins and ECM in leukocyte development (for review see Hemler, 1990) and in neural development and migration (for review see Reichardt and Tomaselli, 1991) has been clearly demonstrated. Integrins bind certain components of the extracellular matrix, mainly glycopro-

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1. *Abbreviations used in this paper*: AGM, astroglial matrix; CNP, cyclic nucleotide phosphodiesterase; CNS, central nervous system; ECM, extracellular matrix; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; PLP, proteolipid protein; PNS, peripheral nervous system.

teins such as fibronectin, laminin, and vitronectin (for review see Hynes, 1987; Ruoslahti and Pierschbacher, 1986). The integrins were originally classified into three major subfamilies (β_1, β_2, and β_3) each having a common β subunit noncovalently associated with a distinct set of α subunits (Hynes, 1987). In addition to these three well characterized β subunits, there have been five other β subunits reported including β_4 (Kajiji et al., 1989; Suzuki and Naitoh, 1990; Hogervorst et al., 1990), β_5 (Ramaswamy and Hemler, 1990; McLean et al., 1990), β_6 (Sheppard et al., 1990), β_7 (Yuan et al., 1992; Erle et al., 1991), and β_8 (Moyle et al., 1991). It has also been shown that some α subunits can associate with more than one β subunit and therefore there is no longer a clear demarcation between subfamilies (Cheresh et al., 1989; Kajiji et al., 1989; Vogel et al., 1990; Krissansen et al., 1990; Dedhar and Gray, 1990).

Some integrins (e.g., the fibronectin receptor) recognize the tripeptide sequence RGD (Arginine-Glycine-Aspartic Acid) which appears to play a key role in cell adhesion (for review see Ruoslahti and Pierschbacher, 1987). Our laboratory previously reported that isolated oligodendrocytes are able to bind to components of a matrix derived from glial cells in culture via a protein which appeared to have integrin-like binding properties (Cardwell and Rome, 1988*a*). In this report we have investigated the biochemical nature of this protein and present direct evidence that it is a member of the integrin superfamily. In addition, this receptor appears to play a regulatory role in CNS myelination.

Materials and Methods

Cell Culture and Astroglial Matrix Preparation

Purified oligodendrocytes were prepared from neonatal rat cerebral cortex after the method of McCarthy and de Vellis (1980) with modifications (Rome et al., 1986; Cardwell and Rome, 1988*a*). Two days after isolation, greater than 80% of the cells stain positively for the oligodendrocyte marker, galactocerebroside, and most cells that score as negative for galactocerebroside possess oligodendrocyte morphology, and, in the presence of 5% calf serum-containing medium, go on to express galactocerebroside with time in culture (Cardwell and Rome, 1988*a*). Astroglial matrix (AGM) was prepared as described earlier (Rome et al., 1986; Cardwell and Rome, 1988*a*). Briefly, mixed glial cells were cultured in 100-mm tissue culture plates and grown to confluence. Media was then removed and 10 ml of distilled water was added to each plate. After 2 h or longer incubation at room temperature, the lysed cell material was removed. The plates were washed two times with PBS, once with serum-free medium, and stored in medium at 37°C until use. The material remaining on the culture surface after water lysis is referred to as AGM (astroglial matrix). Typical AGM contained 0.5-1 μg protein per cm^2 surface.

We use the term equivalent "brain age" to mean the days in culture plus the age of the rat pups at the time of dissection (2 d in these studies). Isolated oligodendrocytes (10–12 d equivalent brain age) were either used for surface labeling or plated onto AGM-coated tissue culture plates and maintained in DMEM/F12 (1:1) containing Hepes (15 mM, pH 7.1), $NaHCO_3$ (1.2 g/l), and 5% calf serum (Hyclone, Logan, UT) for RNA preparation. Astrocytes, prepared by the method of McCarthy and de Vellis (1980), were maintained in DMEM/F12 as above. Rat and human skin fibroblasts were established in our laboratory and cultured in low glucose DMEM medium supplemented with 10% calf serum.

Cell Surface Labeling and Immunoprecipitation

For surface-labeling, oligodendrocytes from 80 cortices (from 40 neonatal rat pups) were removed from mixed glial cells by overnight shaking (McCarthy and de Vellis, 1980) and pelleted by brief centrifugation in a table-top centrifuge at 450 *g*. The pellet (5×10^7 cells) was washed twice with PBS by resuspension and centrifugation and the final pellet was suspended in 1 ml PBS containing 1 mM Ca^{++} and 1 mM Mg^{++}. Astrocytes (10^8 cells) and fibroblasts (5×10^7 cells) were removed from flasks by incubating in 20 mM EDTA in PBS (2 ml per flask) for 10–20 min. Detached cells were collected in PBS containing 1 mM each Ca^{++} and Mg^{++} and pelleted by centrifugation at 450 *g*. Cells were washed three times and resuspended in 1 ml of the same buffer. Iodination of the cells was performed using the iodogen method (Markwell and Fox, 1978). Surface-iodinated cells were extracted on ice with Tris buffer (100 mM Tris-HCl, 0.15 M NaCl, 0.5 mM $MgCl_2 \cdot 6H_2O$) pH 7.2, containing 0.5% NP-40, 0.1% aprotinin, 1 mM PMSF, and 1 μM pepstatin A (Sigma Chem. Co., St. Louis, MO). The extracts were clarified by centrifugation at 14,000 *g* for 15 min followed by incubation with non-immune rabbit or mouse serum and protein A–Sepharose beads (anti–mouse IgG-agarose for mouse serum). Integrin heterodimers were then immunoprecipitated using one or more of the following polyclonal antibodies raised against either an intact integrin subunit (anti-β_1), or a synthetic peptide of the cytoplasmic domain (anti-β_3, anti-β_5, anti-α_3, anti-α_4, anti-α_5, anti-α_8, anti-α_9 anti-αv subunits) or a monoclonal antibody (anti-α_7). The antibodies were generous gifts of Dr. Martin Hemler (anti-β_1, anti-β_5, anti-α_4), Dr. Richard Hynes (anti-β_3, anti-α_3, and anti-α_5), Dr. Stephen Kaufman (anti-α_7), Drs. Lynn Schnapp and Robert Pytela (anti-α_8), Dr. Dean Sheppard (anti-α_9), and Dr. Louis Reichardt (anti-αv). The immune complexes were recovered with protein A–Sepharose or anti–mouse IgG-Agarose. After extensive washing, immune complexes were dissociated from the beads by boiling in sample buffer (2% SDS, 100 mM Tris-HCl, pH 6.8, 10% glycerol, 10 mM EDTA). Samples were analyzed by SDS-PAGE using 4% and 6% acrylamide in the stacking and running gels, respectively (Laemmli, 1970). Gels were then dried, and exposed to Kodak XAR-5 film to visualize labeled integrin subunits. All immunoprecipitation results were confirmed by a minimum of three repeat experiments.

Treatment of Integrins with N-Glycanase F

Oligodendrocyte and fibroblast integrins were immunoprecipitated using anti-β_1 antibody as described above. Immunoprecipitated protein was denatured by boiling in 1% SDS for 3 min. Sodium phosphate buffer (20 mM, pH 7.2) containing 10 mM sodium azide, 50 mM EDTA, and 0.5% n-octylglucoside was then added to bring the SDS concentration to 0.1% and the samples boiled again for 3 min. After cooling, N-glycosidase F (0.5 unit; Boehringer Mannheim Corp., Indianapolis, IN) was added to each sample followed by incubation for 16 h at 37°C before analysis on SDS-PAGE.

Northern Blot Analysis

Oligodendrocytes (10 d equivalent brain age) were plated onto 150-mm tissue culture plates coated with AGM (see above). At 17 d equivalent brain age, select cultures were treated for a period of 48 h with 0.1 mg/ml GRGDSP peptides, or 0.1 mg/ml GRGESP peptides, or 0.1 μg/ml cycloheximide, or 0.1 mg/ml GRGDSP + 0.1 μg/ml cycloheximide. Total RNA was prepared by the method of Chomczynski and Sacchi (1987) using acid guanidinium thiocyanate-phenol-chloroform extraction. For developmental studies, isolated oligodendrocytes were plated in 150-mm tissue culture plates and RNA was prepared from each culture at various stages of development. The earliest time point was at day 13 and the latest was at day 23 (equivalent brain age). RNA separation was carried out on 1.0% agarose/formaldehyde gels before transfer to nylon membranes (ICN Biotrans). Blots were prehybridized in 50% formamide, 0.2% SDS, 5× Denhardt's, 5× Pipes, and 10 μg/ml salmon sperm DNA overnight at 42°C. Hybridization probes (myelin basic protein [MBP], proteolipid protein [PLP], and cyclic nucleotide phosphodiesterase [CNP] and human β_1 integrin, generous gifts of Drs. Anthony Campagnoni, Robert Milner, Sally Lewis, and Erkki Ruoslahti, respectively) were labeled with [α-^{32}P]dCTP to a specific activity of $\sim 1.2 \times 10^9$ cpm/μg by the random priming method (BRL). Hybridization was carried out overnight at 42°C in the same solution as prehybridization buffer except Denhardt's was used at 1× and salmon sperm DNA was added to 100 μg/ml. After hybridization, blots were washed two times (1 h each) in 2× SSC containing 0.2% SDS for 1 h at 42°C followed by a third wash in 2× SSC, containing 0.2% SDS at 56°C for 1 h and exposed to Kodak XAR-5 film. To normalize for the amount of RNAs loaded in each well, blots were stripped and reprobed for chicken β-actin (Cleveland et al., 1980). Autoradiographs were scanned with an Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology, Piscataway, NJ) and analyzed with the GelScan XL 2.1 software package (Pharmacia LKB).

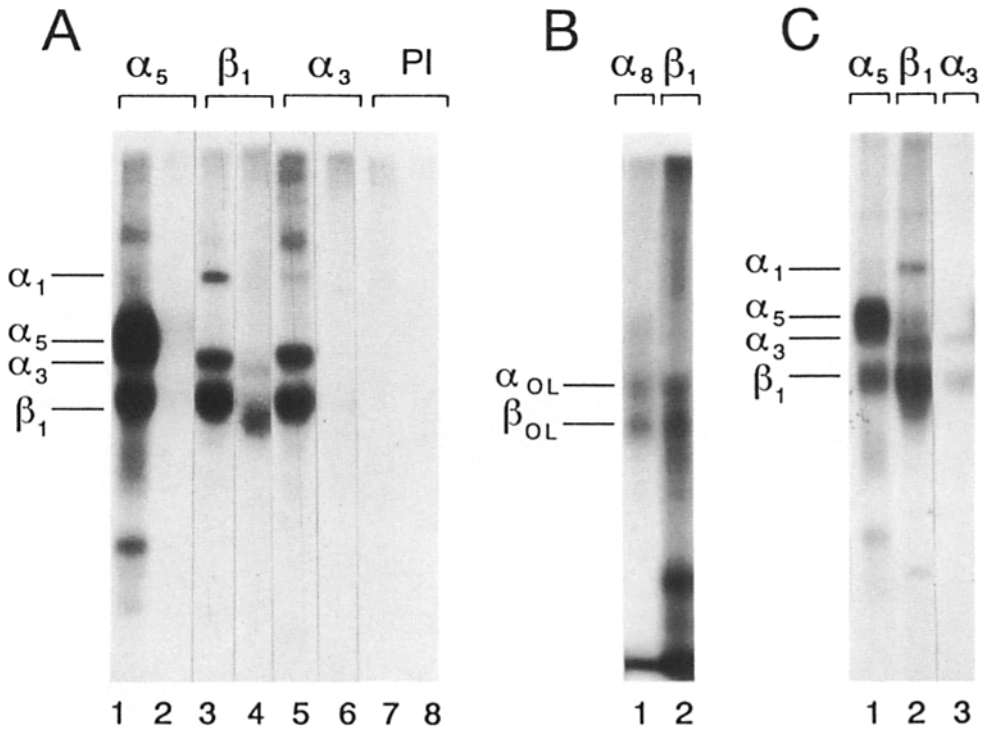

Figure 1. Immunoprecipitation from surface-iodinated fibroblasts (*A*, lanes *1*, *3*, *5*, and *7*), oligodendrocytes (*A*, lanes *2*, *4*, *6*, and *8*; *B*, lanes *1* and *2*), and astrocytes (*C*, lanes *1–3*) using antibodies to α_5, β_1, α_3, and α_8 integrin subunits as well as preimmune control antibody (*PI*) as indicated on the figure. The experiments in *A* and *C* represent sequential immunoprecipitations on each cell extract using the indicated antibodies. The precipitates were analyzed under nonreducing conditions by SDS-PAGE. The three panels represent three different SDS gels; however, the positions of the integrin chains have been labeled consistently throughout with respect to molecular weight standards on each gel.

Results

Immunochemical Analysis

To demonstrate directly the existence of integrins on the surface of rat oligodendrocytes, surface iodinated cells were solubilized and immunoprecipitated using antibodies to various integrin subunits. Antibodies specific for two different α chains (α_3, and α_5) and three different β chains (β_1, β_3, and β_5) were initially tested. Only one antibody, anti-β_1, gave a positive reaction with the labeled oligodendrocytes. In contrast, anti-α_3, α_5, and β_1 were all able to precipitate integrin subunits from detergent-solubilized surface labeled rat fibroblast samples run in parallel as a control. As indicated in Fig. 1 *A*, under non-reducing conditions, antiserum to a peptide representing the cytoplasmic domain of the human α_5 integrin subunit coprecipitated an α_5 and an associated β_1 chain from surface-iodinated fibroblasts (Fig. 1 *A*, lane *1*) while the same antiserum failed to detect any immunoreactive material in the detergent solubilized extract of surface-iodinated oligodendrocytes (Fig. 1 *A*, lane 2). Similarly, antiserum to a peptide representing the cytoplasmic domain of chicken α_3 integrin subunit immunoprecipitated an α_3 and associated β_1 from fibroblasts (Fig. 1 *A*, lane *5*). However this antiserum also failed to detect any immunologically cross-reactive protein in the oligodendrocyte lysate (Fig. 1 *A*, lane *6*). In contrast, antiserum against human β_1 integrin coprecipitated two polypeptides, a putative β and an associated α subunit from oligodendrocytes (Fig. 1 *A*, lane *4*); we will refer to these polypeptides as α_{OL} and β_{OL} (OL, for oligodendrocytes). The β_{OL} subunit had lower electrophoretic mobility in SDS-PAGE under non-reduced conditions (Fig. 1 *A*, lane *4*) than β_1 from fibroblasts (Fig. 1 *A*, lanes *3* and *5*). In addition, oligodendrocytes maintained for two weeks in culture expressed this same arrangement of integrin chains (α_{OL} and β_{OL}), indicating that the pattern of integrin expression was unchanged between days 12 and 21 equivalent brain age.

As mentioned above, we also tested the possibility of expression by oligodendrocytes of other β subfamily integrins such as β_5 and β_3. Both antibodies failed to detect any immunoreactive material in the oligodendrocytes lysate (data not shown). In addition, we examined whether the α_{OL} chain could be α_4, or α_V two other subunits that have been reported to associate with β_1 (Hemler et al., 1987; Vogel et al., 1990). Both anti-α_4 and α_V antibodies were found to be unreactive with the surface-labeled oligodendrocyte extracts (data not shown). While this work was in process, we obtained antibodies raised against integrin subunits α_7, (Song et al., 1992), α_8 (L. Schnapp and R. Pytela, personal communication), and α_9 (Palmer et al., 1993), the more recently identified α subunits that appear to associate with a β_1 chain. Immunoprecipitation of an ^{125}I-labeled extract of oligodendrocytes using the above antibodies showed that only the α_8 antibody reacted with the labeled extract (Fig. 1 *B*). Two polypeptides were precipitated, a putative α that has a smaller size from that reported for chick α_8 (Bossy et al., 1991) and an associated β subunit (Fig. 1 *B*, lane *1*). The protein doublet immunoprecipitated with anti-α_8 had a mobility in SDS-PAGE identical to the doublet immunoprecipitated with anti-β_1 (α_{OL}, β_{OL}) (Fig. 1 *B*, lanes *1* and *2*, respectively) suggesting that α_{OL} could be α_8 or an α_8-related subunit.

Expression of integrins by rat astrocytes, the other major glial cell type in the CNS, was also examined using some of the same antibodies as above to immunoprecipitate extracts of surface-iodinated cells. An antibody to the α_5 integrin subunit coprecipitated and an α and an associated β subunit (Fig. 1 *C*, lane *1*). The position of migration of these two bands corresponds to α_5 and β_1 from rat fibroblasts (Fig. 1 *A*, lane *1*). Similarly, antibody to α_3 integrin coprecipitated

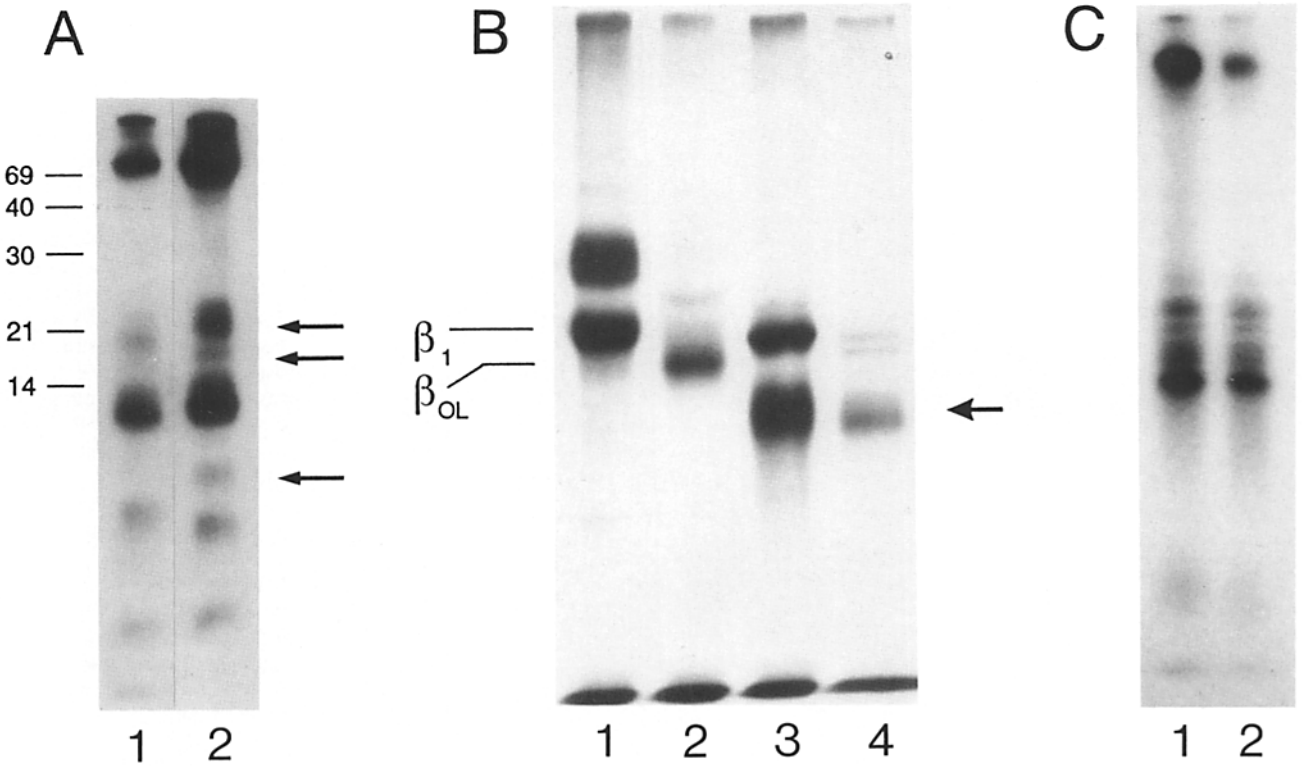

Figure 2. Comparison of rat oligodendrocyte β_{OL} and rat fibroblast β_1 by one-dimensional peptide mapping with V8 protease. ^{125}I-labeled integrins from detergent-solubilized extracts of oligodendrocytes and fibroblasts were immunoprecipitated with antibodies raised to human β_1 integrin; individual subunits were separated by SDS-PAGE. (*A*) Gel slices containing β_{OL} (lane *1*) and β_1 (lane *2*) were each treated with 1 μg V8 protease during a second electrophoresis on a 15% SDS-polyacrylamide gel (Cleveland et al., 1977). Arrowheads indicate the differences in peptide maps of β_1 and β_{OL}. Numbers at the left indicate the position and size in kD of molecular weight markers. (*B*) Integrin subunits were immunoprecipitated from rat fibroblasts and oligodendrocytes by antisera raised against human β_1 and either remained as control (lanes *1* and *2*) or treated with N-glycanase F (lanes *3* and *4*, see Materials and Methods). Arrowhead indicates position of migration of β_1 (lane *3*) and β_{OL} (lane *4*) after deglycosylation. Samples were analyzed under nonreducing conditions by SDS-PAGE. (*C*) Comparative peptide maps of fibroblast β_1 (lane *1*) and oligodendrocyte β_{OL} (lane *2*) integrin subunits after deglycosylation by N-glycanase F.

an α and an associated β (Fig. 1 *C*, lane *3*) with identical mobility to α_3 and β_1 from rat fibroblasts (Fig. 1 *A*, lane *3*). It appears that astrocytes express a relatively lower level of α_3 compared to fibroblasts. Antibody to β_1 integrin also precipitated a β subunit and associated α_1, α_3, and α_5 chains (Fig. 1 *C*, lane *2*). The β_1 subunit precipitated from astrocytes ran as a broad band (Fig. 1 *C*, lane *2*), this was likely due to both overloading and to the presence of a small amount of β_{OL} subunit which could arise from the ~5% oligodendrocyte contamination commonly seen in astrocyte preparations (this latter hypothesis was supported by repeat experiments on astrocytes where the lower molecular weight material was not observed).

Chemical Characterization of the Oligodendrocyte Integrin

The β_{OL} polypeptide was further compared to the putative fibroblast β_1 chain by one dimensional peptide mapping (Cleveland et al., 1977). Both integrin β_{OL} and β_1 subunits yielded several identical peptide fragments (Fig. 2 *A*, compare lanes *1* and *2*), as well as distinct fragments (Fig. 2 *A*, *arrows*). In the 18–30-Kd region, there are 2–3 fragments generated from the fibroblasts β_1 while in the same region β_{OL} produced only one peptide fragment that did not comigrate with any of those from fibroblasts (see *arrows*). In the region below 14 Kd, the β_1 digest has an additional peptide that is missing in the β_{OL} digest (*arrow*). There was too little material from the α_{OL} sample to generate a distinct map.

To test whether differential glycosylation accounts for the differences in molecular weight and peptide maps of the fibroblast β_1 and oligodendrocyte β_{OL} chains, the polypeptides were treated with N-glycanase F to remove N-linked carbohydrate chains. After digestion, the two β subunits ran as smaller proteins which comigrated in a non-reduced SDS gel (Fig. 2 *B*, lanes *3* and *4*, β_1 and β_{OL}, respectively, *arrow*). The α chains were also reduced in size but did not appear to run at identical mobilities. The deglycosylated α_{OL} chain ran as a closely spaced doublet in this experiment, likely due to incomplete deglycosylation, since in other experiments only a single band at the lower size was seen (not shown). Peptide maps of the deglycosylated β chains were generated and found to be identical (Fig. 2 *C*).

Developmental Expression of β_{OL} mRNA

The β_{OL} and β_1 subunits were also highly related at the mRNA level, Northern blots of total oligodendrocyte mRNA probed with a full-length cDNA specific for the human β_1 integrin subunit, revealed a single 3.2-Kb message (Fig. 3,

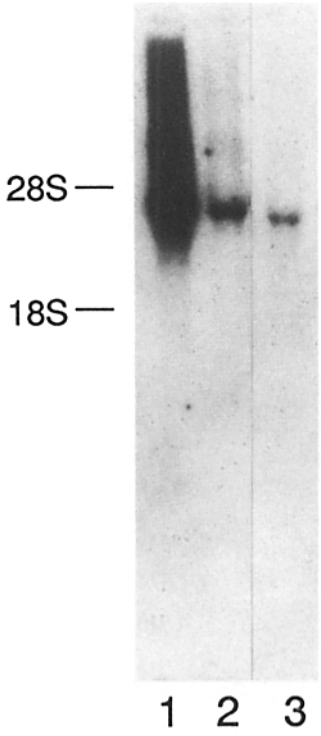

Figure 3. Northern blot analysis of integrin β subunit message expressed by oligodendrocytes. Rat and human fibroblasts were used as control cells. Total mRNA from human fibroblasts (lane *1*, 10 μg), rat fibroblasts (lane 2, 10 μg) and rat oligodendrocytes (lane *3*, 30 μg) were prepared, transferred to a nylon membrane, and probed with a human β_1 integrin cDNA as described in Materials and Methods.

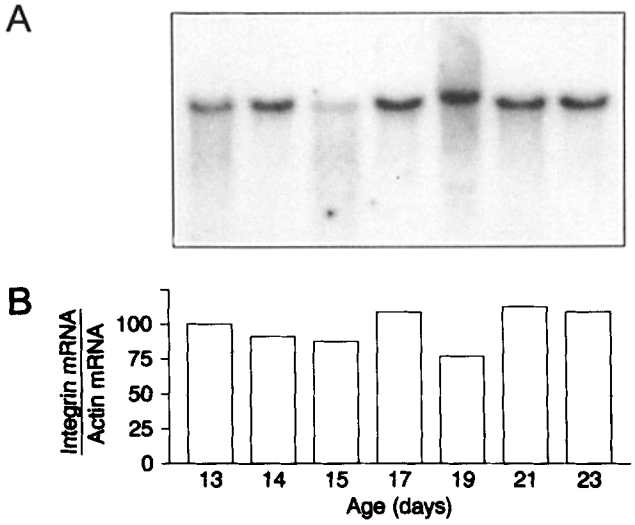

Figure 4. Expression of oligodendrocyte integrin β subunit (β_{OL}) during maturation of oligodendrocytes. (*A*) Total mRNA was isolated from purified oligodendrocytes at different developmental stages and analyzed by Northern blots using rat β_{OL} integrin cDNA (see results). (*B*) Densitometric quantitation of mRNAs in *A* relative to β-actin probed in the same gel (not shown). This experiment was repeated twice with essentially the same result.

lane *3*). We used the human probe since to our knowledge, the rat β_1 has not been cloned. This message was approximately the same size as that seen in both human and rat fibroblast mRNA (Fig. 3, lanes *1* and *2*, respectively). We recently isolated a putative β_{OL} cDNA from an oligodendrocyte cDNA library (Malek-Hadayat, S., and L. H. Rome, manuscript in preparation). This clone shows >90% identity to mouse β_1 cDNA and detects the same size oligodendrocyte mRNA in a Northern blot (not shown).

To examine whether expression of β_{OL} mRNA is developmentally regulated, total mRNA was prepared from isolated oligodendrocyte cultures at various developmental stages between day 13 (the earliest age at which we can obtain pure cells) and day 23 (a time beyond the peak period of myelin synthesis). The mRNAs were analyzed by Northern blots using the β_{OL} cDNA as a hybridization probe. Results shown in Fig. 4 *A* indicated no significant differences between the levels of mRNA expressed. This was confirmed by densitometric quantitation relative to β-actin expression which was probed in the same gel (Fig. 4 *B*).

Effect of GRGDSP Peptides and Cycloheximide on Expression of Integrin and Myelin-Specific Messages by Oligodendrocytes

We have previously reported that GRGDSP synthetic peptides can block the initial attachment of oligodendrocytes to their substratum, AGM (Cardwell and Rome, 1988*a*). However, the addition of GRGDSP peptides to established oligodendrocyte cultures does not cause cell detachment, yet these peptides significantly reduced the synthesis of MBP (Cardwell and Rome, 1988*b*). To further analyze the mechanism of action of the GRGDSP peptides, we examined the effect of these peptides on the level of expression of mRNAs for several myelin genes, including MBP, CNP, and PLP. The results were compared to mRNA expression in the presence of a control non-specific peptide, GRGESP, and normalized to expression of β-actin. Cells grown in the presence of 0.1 mg/ml GRGDSP peptides showed a 74% reduction in

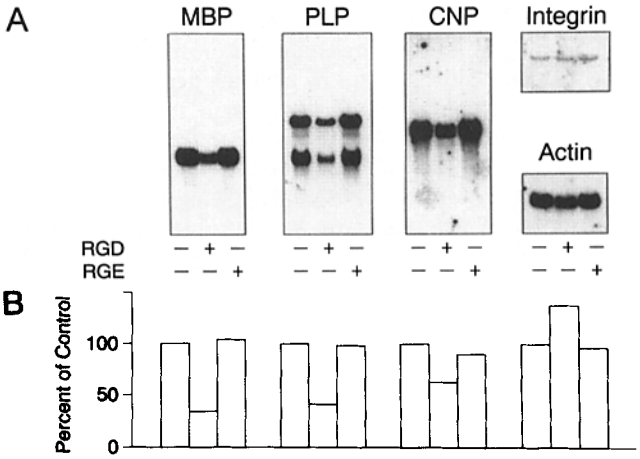

Figure 5. (*A*) Effect of GRGDSP and GRGESP synthetic peptides on expression of mRNAs for MBP, CNP, PLP, and integrin (β_{OL}) by oligodendrocytes. Purified oligodendrocytes cultured on AGM (control, *first lane each probe*), or treated for 48 h with 0.1 mg/ml GRGDSP (*center lane each probe*), or 0.1 mg/ml GRGESP (*third lane each probe*). Total mRNAs were extracted, separated on a 1% agarose gel (12 μg per well) and transferred to a nylon membrane. Blots were probed with ^{32}P-labeled MBP, PLP, CNP, oligodendrocyte integrin β_{OL}, and β-actin cDNAs as described in Materials and Methods. (*B*) mRNAs from each blot in 5A were quantitated by densitometry and normalized to the amount of β-actin in each lane. This experiment was repeated several times with the following results (mean $\pm$ SD): the inhibition of mRNA expression by GRGDSP was 74 $\pm$ 7% for MBP (n = 3), 64 $\pm$ 7% for PLP (n = 3), and 44 $\pm$ 8% for CNP (n = 2).

Table I. Effect of GRGDSP and Cycloheximide on Expression of mRNAs for MBP and PLP by Oligodendrocytes

Conditions*	MBP	PLP
	% of Control‡	% of Control‡
Control	100	100
+ 0.1 mg/ml GRGDSP	22	28
+ 0.1 mg/ml GRGDSP + 0.1 μg/ml cycloheximide	78	61
+ 0.1 μg/ml cycloheximide	92	106

* Purified oligodendrocytes were cultured on AGM alone (control) or treated for 48 h with GRGDSP, GRGDSP + cycloheximide, or cycloheximide alone. Total mRNAs were extracted, separated on a 1% agarose gel (20 μg per well) and transferred to a nylon membrane. The membrane was sequentially probed with ^{32}P-labeled MBP, PLP, and β-actin cDNAs as described in Materials and Methods.
‡ Values were calculated from densitometric scans and normalized to the relative density of β-actin.

the amount of MBP mRNA relative to untreated control cells (Fig. 5, *A* and *B*, MBP, lanes *2* and *1*, respectively). In rat, PLP message is expressed as two different species of 1.6 and 3.2 Kb, both messages were reduced by ~64% compared to control (Fig. 5, *A* and *B*, PLP, lanes *2* and *1*, respectively). Similarly, the CNP mRNA was decreased by ~40% relative to control (Fig. 5, *A* and *B*, CNP, lanes *2* and *1*, respectively). In contrast, the control peptide, GRGESP, showed no significant inhibitory effect on expression of any of the myelin genes (Fig. 5, third lane for each probe). In all experiments the level of peptide added (0.1 mg/ml) did not result in a significant detachment of cells from the culture substratum (less than 3% of the cells detached). The effect of RGD-containing peptides on expression of β_{OL} was also examined. In contrast to the myelin genes, Northern blot analysis showed that RGD-containing peptides had no effect on the level of this putative β_{OL} mRNA (Fig. 5, *A* and *B* as indicated), which supports the selective regulation of myelin genes by RGD-containing peptides.

Inhibition by RGD-containing peptides could be a direct effect on transcription of myelin genes or the peptides could be acting indirectly, perhaps by affecting genes encoding intermediary acting factors. To differentiate these two mechanisms, we treated cells with GRGDSP in the presence of cycloheximide (Table I). Cycloheximide blocked the GRGDSP inhibition to a significant extent, allowing maintenance of MBP, and PLP messages near the control levels. Cycloheximide alone did not super-induce message for either of the myelin proteins (Table I).

Discussion

In this study we have described the expression of a single integrin receptor complex by rat cerebral cortex oligodendroglia, a cell type restricted to the central nervous system and responsible for the synthesis of CNS myelin. Evidence presented here indicate that this integrin is an alternately glycosylated member of the β_1 subfamily. Preliminary results suggest that the associated α subunit is α_8, however, definitive proof will require additional chemical and/or molecular analysis.

In addition to the chemical characterization, we have also presented evidence for a potential regulatory role for this receptor in synthesis of myelin components. We previously reported that isolated oligodendroglia in primary culture interact specifically with matrix components derived from mixed glial cells. Moreover, a synthetic hexapeptide containing the RGD sequence disrupts this interaction and inhibits the synthesis of myelin components such as MBP and sulfatides by oligodendrocytes (Cardwell and Rome, 1988*a*,*b*). These results prompted us to study the nature of this interaction and its effect on myelination. Based on the RGD and divalent cation dependence of oligodendrocyte adhesion, we speculated that a likely candidate for the adhesion receptor could be a member of the integrin family of receptors for ECM proteins. In the present study we used antibodies raised against several α and β integrin subunits to probe for the presence of an oligodendrocyte integrin. Rat fibroblasts were used as control cells since these cells are known to express a number of integrin chains including $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$ (Malek-Hedayat and Rome, 1992). Of nine antibodies tested, only anti-β_1 and anti-α_8 were able to immunoprecipitate an integrin complex from oligodendrocytes. Furthermore, the complexes immunoprecipitated with both antibodies were strikingly similar to each other with respect to electrophoretic mobility on non-reduced SDS-PAGE. Using a similar battery of antibodies we found that astrocytes, the other major glial cell type in brain, express multiple integrin receptors including $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$. This combination of integrin chains is also expressed in C6 glioma cells, a chemically induced tumor cell line from rat brain (Malek-Hedayat and Rome, 1992). Using a monoclonal antibody (3A3), which recognizes an $\alpha_1\beta_1$ heterodimer, Tawil et al. (1990) previously demonstrated the presence of this integrin on the surface of rat astrocytes. Astrocytes from mouse can be stained with anti-fibronectin receptor antibodies (Pesheva et al., 1988), which is consistent with our finding of $\alpha_5\beta_1$ in rat astrocytes.

The oligodendrocyte integrin β subunit (β_{OL}) and the β_1 subunit expressed by rat fibroblasts displayed different mobilities on non-reducing SDS-PAGE. However, peptide maps of the two subunits indicated that they were highly related. After removal of N-linked carbohydrate chains by N-glycanase F both subunits were found to comigrate on SDS gels. In addition, peptide maps of the two deglycosylated subunits were indistinguishable, suggesting that β_{OL} and β_1 are identical at the amino acid level and that differential glycosylation occurs in oligodendrocytes and fibroblasts. Due to the unique role of the oligodendrocyte integrin in regulation of myelin synthesis, it is possible that this cell-specific glycosylation may play a role in receptor function. A number of studies have recently attempted to examine the role of integrin carbohydrate chains on the adhesive properties and biological function of these receptors. During development, mouse T cells have been shown to express two different β_1 subunits that differ in the extent of N-linked glycosylation and sialylation. The differential glycosylation of the β_1 subunit appears to effect binding of the receptor to fibronectin (via VLA-4 and VLA-5) and laminin (via VLA-6) (Wadsworth et al., 1993). The effect of an altered glycosylation

of the β_1 subunit on binding of the cells to fibronectin and laminin has been shown as well by other investigators (Akiyama et al., 1989; Kawano et al., 1993; Diamond et al., 1991; Öz et al., 1989). It remains to be determined whether the altered glycosylation of β_{OL}, compared to its rat fibroblast homolog, plays a significant role in receptor function.

We have not characterized the α_{OL} subunit to the same extent as β_{OL}. It appears to be either expressed in lower abundance or less efficiently iodinated than β_{OL}. Our preliminary results using antibodies to a number of recently described α subunits suggest that α_{OL} might be α_8 since antibody raised against the cytoplasmic domain of human integrin α_8 subunit immunoprecipitated two polypeptides from oligodendrocytes with the identical mobilities on SDS-PAGE as $\alpha_{OL}\beta_{OL}$ (Fig. 1 *B*). However, attempts to produce definitive comparative peptide maps of α_{OL} and α_8 have been thus far unsuccessful.

The possibility that occupancy of the $\alpha_{OL}\beta_{OL}$ integrin by RGD-containing peptides inhibits myelin synthesis by oligodendroglia suggests an important functional role for this receptor that may go beyond cell-substratum adhesion. There are now numerous examples in the literature where binding to extracellular matrix has been shown to regulate transcriptional activity and thus alter cellular differentiation. One of the first examples was in myoblasts, where anti-integrin antibodies were shown to prevent cell differentiation into myotubules (Menko and Boettinger, 1987). We have added anti-β_1 antibodies to isolated oligodendrocytes in an attempt to mimic the inhibition of myelination that results from addition of RGD-containing peptides. Three different anti-β_1 polyclonals have been tested without success. However, these function-blocking experiments are complex, requiring antibodies against native determinants at or near functional extracellular domains.

It is important to stress that the evidence we present here for a functional role of the oligodendrocyte β_1-related integrin in myelination is indirect. The evidence, presented here and in previous studies (Cardwell and Rome, 1988*a*,*b*) includes (*a*) the presence of a single detectable integrin receptor on oligodendrocytes using a wide variety of anti-α and β chain antibodies; (*b*) the specific inhibition of oligodendrocyte adhesion by RGD-containing peptides; and (*c*) the specific inhibition of myelin synthesis by these same peptides (including reduction in mRNAs for the major myelin components, and a reduction in the synthesis of myelin lipids and proteins). Without a function-blocking anti-integrin antibody or other direct data, one must exercise caution in concluding that the RGD effects on myelin synthesis are mediated through the oligodendrocyte integrin. However, we feel that this data supports the hypothesis that specific adhesion of oligodendrocytes to a glial-derived matrix via an integrin receptor regulates myelin gene expression and thereby plays a critical role in differentiation of oligodendrocytes into a myelinating phenotype. A dissection of the biochemical events that lead to such a regulatory response has yet to be carried out, however, based on the present results it is reasonable to hypothesize that an integrin-mediated adhesion of oligodendrocytes triggers signal(s) that directly or indirectly induce the expression of myelin genes. This hypothesis is supported by the observation that cycloheximide caused a significant reduction in the inhibitory effect of RGD peptides (Table I), suggesting that a new protein must be synthesized for the inhibition to occur. Cycloheximide is also known to selectively stabilize certain mRNAs, however, the absence of increased mRNA levels in the presence of cycloheximide alone, argues against this being the mechanism of cycloheximide action. Although it is still possible that RGD peptides cause myelin mRNAs to become unstable and that this instability is overcome by the addition of cycloheximide. The involvement of integrins in signal transduction is a relatively recently described phenomenon for which there is now increasing evidence (for review see Hynes, 1992). For example, it has been reported that in human neutrophils (Jaconi et al., 1991; Richter et al., 1990) and osteoclasts (Miyauchi et al., 1991) changes in intracellular Ca^{++} occur in response to adherence of integrin receptors to matrix components. In addition other integrin-linked signaling events such as changes in cAMP levels (Nathan and Sanchez, 1990), increased protein tyrosine phosphorylation (Kornberg et al., 1991) and protein kinase C-dependent cytoskeletal rearrangement (Pardi et al., 1992) have been reported. Involvement of integrins in regulation of gene expression in rabbit synovial fibroblasts has also been studied (Werb et al., 1989).

If the oligodendrocyte integrin we describe here does in fact regulate myelin expression in vivo, we might expect its level of expression to change during glial cell development. Thus far we have examined β_1 expression in oligodendrocytes only over a limited period (13–21 d) without detecting a significant change in mRNA level. However, changes in β_1 mRNA expression could occur earlier than day 13 or alternatively, the expression of the α chain may be regulated.

We are now in the process of determining the integrin-mediated signaling events that regulate myelin synthesis. The matrix target of the oligodendrocyte integrin also remains an important issue. Despite continued efforts, the biochemical nature of the AGM ligand recognized by the oligodendrocyte integrin is still unknown. Antibodies to several known ECM proteins including fibronectin, laminin, tenascin, and vitronectin failed to inhibit oligodendrocytes adhesion to AGM (Cardwell and Rome, 1988*a*; Malek-Hedayat, S., and L. H. Rome, unpublished observations). However, the AGM ligand appears to be highly insoluble since the adhesive activity can not be removed from AGM by strong detergents, chaotropic agents, high salt, or low pH (Hamilton, S. P., and L. H. Rome, unpublished observations). It is possible that the AGM ligand recognized by the oligodendrocyte integrin described here is a novel ECM component. In light of our preliminary results with anti-α_8 antibody, it is intriguing that the identity of ligand(s) for α_8-containing integrins is still unknown (Bossy et al., 1991).

We are grateful to Hedi Roseboro and Dion Baybridge for their expert technical assistance and to Drs. Wendy B. Macklin, Diane Chugani, and Martin Hemler for their helpful suggestions. We would also like to thank Drs. Richard Hynes, Martin Hemler, Stephen Kaufman, Dean Sheppard, Lynn Schnapp, Robert Pytela, Erkki Ruoslahti, Anthony Campagnoni, Robert Milner, Louis Reichardt, and Sally Lewis for their generous gifts of antibodies and cDNA probes.

This work was supported by U.S. Public Health Service (USPHS) grant HD-06576 and a grant from the Multiple Sclerosis Society (RG 2200-A-1). S. Malek-Hedayat was supported by a postdoctoral fellowship from the UCLA Mental Retardation Research Center (USPHS grant HD-07032).

Received for publication 18 August 1993 and in revised form 14 December 1993.

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